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Polarized Domains of Myelinated Axons

Review

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The entire length of myelinated axons is organized into a series of polarized domains that center around nodes of Ranvier. These domains, which are crucial for normal saltatory conduction, consist of distinct multiprotein complexes of cell adhesion molecules, ion channels, and scaffolding molecules; they also differ in their diameter, organelle content, and rates of axonal transport. Juxtacrine signals from myelinating glia direct their sequential assembly. The composition, mechanisms of assembly, and function of these molecular domains will be reviewed. I also discuss similarities of this domain organization to that of polarized epithelia and present emerging evidence that disorders of domain organization and function contribute to the axonopathies of myelin and other neurologic disorders.

Axons exhibit an intimate anatomic and functional relationship with myelinating glia, i.e., Schwann cells in the PNS and oligodendrocytes in the CNS, that is among the most striking of any known cell interactions. Myelinated fibers develop in a series of stages that result from reciprocal, axo-glial interactions. Axon outgrowth precedes and regulates gliogenesis via mitogenic and trophic effects to ensure that glial numbers are matched to axon length (Barres and Raff, 1999; Mirsky et al., 2002). Axons subsequently promote expression of the myelinating phenotype, most strikingly in the PNS, where Schwann cells undergo a major transition in their organization and molecular phenotype that is driven by signals from the axon (Barres, 1998; Jessen and Mirsky, 2002).

Interactions with myelinating glia, in turn, remodel axons, which are initially quite uniform, into highly polarized structures. Voltage-gated sodium (Na^+) and potassium (K^+) channels cluster into distinct domains, the node and juxtaparanode, and are separated by a paranodal junctional complex. The axon cytoskeleton, organelle content, and rates of transport are also specifically modified along the length of myelinated axons. This domain organization is essential for impulse propagation by saltatory conduction, affords the potential for modulating presynaptic input, and is crucial for axon function and integrity. Indeed, axon pathology and disrupted domain organization are increasingly appreciated as sources of morbidity in disorders of myelination. Significant progress in identifying the components, mechanisms of assembly, and function of these domains has been made in recent years and will be described. I also suggest similarities of this organization to that of polarized epithelia. I begin by describing the morphologic organization of myelinated fibers.

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Anatomic Features of Myelinated Axons: Radial and Longitudinal Polarity

In the PNS of vertebrates, Schwann cells either ensheathe multiple individual axons by enclosing them within separate troughs of the cell or myelinate a single axon with which they have established a 1:1 relationship. In general, fibers <1 μm in diameter are unmyelinated and those >1 μm are myelinated (Murray, 1968; Samorajski and Friede, 1968); fibers are typically entirely ensheathed or myelinated along their length, although rare exceptions have been noted (Peters et al., 1991). Myelin sheaths are comprised of multiple wraps of compacted Schwann cell membrane that surround a discrete segment of the axon; the number of lamellae is proportionate to axon diameter (Smith et al., 1982). The nucleus is located on the outside of the sheath (Figure 1). A small collar of cytoplasm persists on the outside of the sheath (the abaxonal compartment) and adjacent to the axon (the adaxonal compartment); the periaxonal space separates the inner glial membrane from the axon. In the PNS, cytoplasmic channels, the Schmidt Lanterman clefts, spiral through the myelin sheath and provide a conduit between the inner and outer cytoplasmic compartments.

In the CNS, axons are myelinated by oligodendrocytes or, in some fiber tracts, lie in direct contact with other nerve fibers or glial cells; there is no equivalent in the CNS to the ensheathing Schwann cell. Oligodendrocytes, unlike Schwann cells, can myelinate multiple axons, forming as many as 40 separate myelin segments. There is considerable heterogeneity in oligodendrocyte morphology, however, as originally noted by del Rio Hortega (1928), and the numbers of myelin segments they form (Butt and Berry, 2000; Remahl and Hildebrand, 1990). An important, additional difference between the CNS and PNS myelin is that a well-defined basal lamina (BL) is associated with the outside of the Schwann cell but not the oligodendrocyte sheath; this BL has a critical role in myelin formation in the PNS (Bunge et al., 1986).

Myelin segments are thus radially polarized. In the PNS, ECM receptors are expressed in the outer (abaxonal) membrane (Previtali et al., 2001), the interior consists of the compact myelin sheath, and the inner (adaxonal) membrane is enriched in adhesion molecules that mediate interactions with the axon, notably the myelin-associated glycoprotein (MAG) (Trapp, 1990). CNS myelin sheaths are also radially organized with distinct proteins in the abaxonal (e.g., oligodendrocyte myelin glycoprotein) and inner glial (e.g., MAG and NCAM) membranes (Salzer, 1995).

Myelinated fibers also exhibit a striking longitudinal polarity that is centered around nodes of Ranvier. These eponymous structures (Ranvier, 1871) are gaps of about 1 μm between each myelin segment where the axon is exposed to and communicates with the extracellular

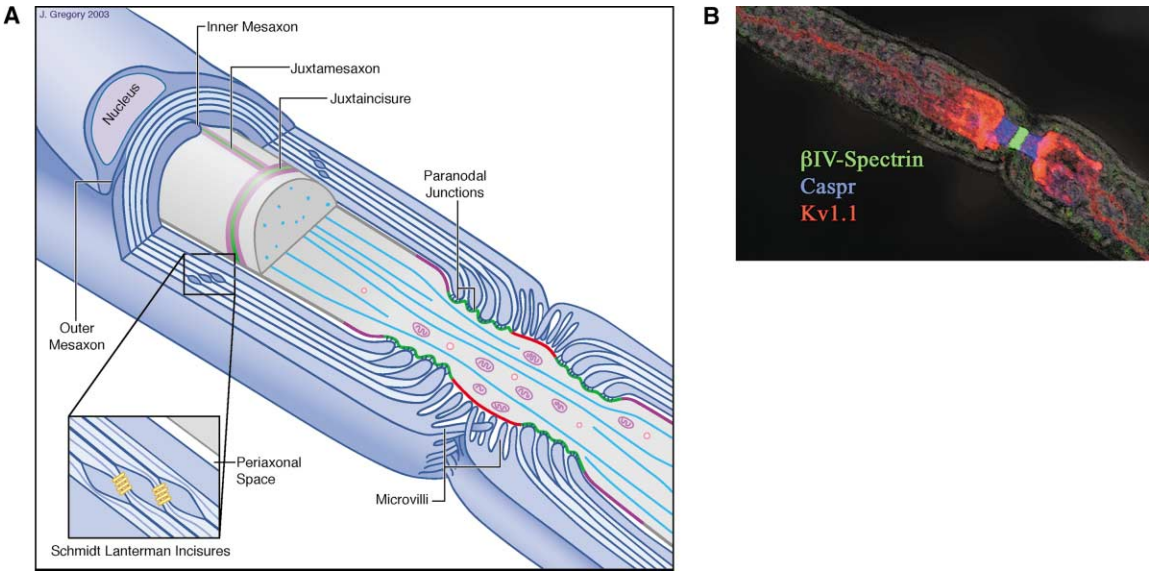

Figure 1. Morphology of Domains of Myelinated Axons (PNS)

(A) Schematic organization of a peripheral myelinated nerve is shown. Cross-section through a myelinated axon (gray) surrounded by two myelin sheaths is illustrated, demonstrating the node of Ranvier (red), to which Schwann cell microvilli project; the paranodal loops and junctions (green); the juxtaparanodal region (purple); Schmidt Lanterman incisures, which form gap junctions (shown at higher magnification in the inset); compact myelin (light blue); and the inner (IM) and outer (OM) mesaxons. The axon diameter is reduced in the region of the node and paranodes, with more tightly packed neurofilaments and accumulation of membrane vesicles and mitochondria. The intranodal axonal specializations, the juxtamesaxon, and juxtaincisures are also shown. The entire structure is surrounded by a basal lamina (not illustrated).
(B) Confocal immunofluorescence of teased sciatic nerve fiber illustrating axonal domains. The node is stained for βIV-spectrin (green), the paranodes for Caspr (blue), and the juxtaparanodes for $K_v1.1$ (red); juxtamesaxonal staining of Kv channels is also seen in the lower right. The field was photographed with DIC to illustrate the overall dimensions of the myelin sheath.

environment. Myelin segments, which extend from node to node, are therefore referred to as internodes; they are approximately 100 times the axon diameter, ranging up to 1 mm or more in larger fibers (Hess and Young, 1952). In the PNS, nodes are contacted by hundreds of interdigitating microvilli that project from the end of the Schwann cell to closely appose the nodal axolemma. Ultrastructural studies suggest continuity between these microvilli and the axonal cytoskeleton (Ichimura and Ellisman, 1991). CNS nodes are devoid of microvilli. Instead, perinodal processes of specialized glia project to many, but not all, nodes (Butt et al., 1999); small fibers in particular lack perinodal processes (Bjartmar et al., 1994). At nodes and initial segments, there is a dense undercoating of the axon membrane that is likely to correspond to a specialized cytoskeleton at these sites (discussed below). There is also an ill-defined nodal gap substance consisting of extracellular matrix molecules. Large fibers, particularly in the PNS, are markedly reduced in diameter at the nodal region (Berthold, 1996).

Nodes are flanked on either side by the lateral edges of myelinating glia, which enclose a continuous belt of cytoplasm that tightly spirals around and forms a unique junction with the axon. In longitudinal sections, this structure has the appearance of a series of loops (up to 40 or more), with the outermost loops closest to the node. These loops closely appose, and physically invaginate, the axolemma; the resultant structure has been compared to a bolt (the axon) threaded into a nut (the paranodal loops) (Peters et al., 1991). The paranodal region is the site of closest apposition between the membranes of the axon and myelinating glia (3–5 nm versus ~15 nm in the internode). Periodic intercellular densities, the transverse bands, are present between each paranodal loop and the axon (Andres, 1965; Bargmann and Lindner, 1964). Consequently, the paranodal junctions are referred to as septate-like based on their morphologic similarities to invertebrate septate junctions (Schnapp et al., 1976). By EM and freeze-fracture, there are approximately six to eight obliquely arranged transverse bands per paranodal loop, each regularly spaced 25–30 nm apart (Peters et al., 1991), suggesting an organized structure imposed by interactions with the cytoskeleton. The transverse bands develop after paranodal loops have attached to the axon (Marcus et al., 2002; Tao-Cheng and Rosenbluth, 1983) and are therefore a hallmark of the mature junction. The paranodal junctions correspond to the largest, continuous intercellular junction known, ranging up to 1 mm^2 in large fibers. As originally proposed by Andres (1965), they provide a paracellular barrier to solute flow between the extracellular space at the nodes and the periaxonal space. In molecular tracer studies, these junctions provide delayed access to the periaxonal space for small molecular weight substances and completely block diffusion of larger molecular weight substances (Hall and Williams, 1971; Hirano and Llena, 1995). By retarding ionic diffusion away from the node, junctions are likely to facilitate saltatory conduction.

In addition to the sepatate-like paranodal junctions, a rich array of reflexive junctions form between the paranodal loops themselves (Spiegel and Peles, 2002). In

both the CNS and PNS, tight junctions seal off the ends of the paranodal loops from the periaxonal space. Autotypic adherens junctions between Schwann cell paranodal loops (Fannon et al., 1995) are likely to provide additional structural integrity to the paranodal region in the PNS. Gap junctions lie between tight junction strands and facilitate transfer of small molecular weight substances between the paranodal loops of Schwann cells. Gap junctions are also present in the Schmidt Lanterman incisures and are important for the long-term integrity of peripheral myelin sheaths (see Scherer and Arroyo, 2002, for further details).

Changes in the Expression of Adhesion Molecules Accompany Myelination

As intially proposed in early electron microscopic studies of peripheral nerve development (Geren, 1954), the myelin sheath is thought to grow by circumferential wrapping of the inner Schwann cell membrane around the axon. In vitro (Bunge et al., 1989) and in vivo (Speidel, 1964) observations of myelin formation support this notion. Loosely spiraled glial membranes are subsequently compacted by extrusion of Schwann cell cytoplasm, except at the edges of the myelin sheath, resulting in the familiar multilamellar organization of the myelin sheath (Webster, 1992). In the CNS, as each oligodendrocyte myelinates multiple axons, topologic considerations mandate that an inner oligodendrocyte membrane similarly wraps the axon to form the multilamellar myelin sheath. In both cases, the myelin sheath expands longitudinally and radially as development proceeds.

These morphogenetic events are associated with significant changes in the expression of cell adhesion molecules on both axons and glia. Many adhesion molecules expressed at high levels at the interface of axons with ensheathing Schwann cells, such as the L1 adhesion molecule, are downregulated with myelination (Martini, 1994). In contrast, MAG (myelin-associated glycoprotein), a glial cell adhesion molecule that promotes association with the internodal axolemma, is strikingly upregulated after an initial turn and a half of myelination (Martini and Schachner, 1986). A key finding is that all known cell adhesion molecules, ion channels, and associated cytoskeletal proteins are initially uniformly expressed along the axon but redistribute into distinct regions of the axon with myelination. These domains are critical for the function of myelinated fibers in propagation of action potentials, as discussed below.

Saltatory Conduction and the Spatiotemporal Integration of Impulses by Myelinated Nerves

Remarkably, the earliest microscopists, including Galvani and Virchow, anticipated that myelinated nerves conducted impulses and that the myelin sheath has insulating properties (Rosenbluth, 1999). However, it was not until 1925 that Lillie first proposed that conduction in myelinated nerves was saltatory and is accelerated by current passing from node to node (Lillie, 1925). Key experimental confirmation was provided subsequently in the 1940s (Huxley and Stämpfli, 1949; Tasaki and Takeuchi, 1941).

The discontinuous nature of saltatory conduction reflects the high concentration of voltage-gated sodium channels at nodes and the low capacitance and high resistance of the myelin sheath. In contrast, the flow of current is continuous in unmyelinated nerves, consistent with the diffuse distribution of ion channels in these fibers (Hille, 2001; Waxman, 1983). Saltatory conduction confers substantial physiologic advantages. In addition to increasing the speed of conduction, nodal conduction results in several thousand-fold economies of energy and space. Thus, on grounds of size and energy utilization, myelination was likely to be essential in the evolutionary development of the more complex and centralized vertebrate nervous system. Key determinants of the speed of propagation are axon diameter and internodal spacing (Waxman, 1980). Theoretical considerations suggest that myelination should enhance conduction velocity only when axon diameters are >1 μm in the PNS (Rushton, 1951) and >0.2 μm or greater in the CNS (Ritchie, 1982), in good agreement with the diameters of myelinated axons in vivo. In general, myelin thickness and internode distance in the PNS correspond to parameters predicted to maximize conduction velocity (Rushton, 1951), suggesting that evolution selected for optimal conduction.

There are notable and informative exceptions to this rule (Waxman, 1997). One example is that internode length is reduced before axon branch points. It can be as short as 30 μm in the preterminal region of peripheral axons (Zenker, 1964), presumably as a mechanism for impedance matching to facilitate invasion of the action potentials into the nonmyelinated terminal regions (Waxman, 1983). In the CNS, myelination is sometimes organized to ensure that axons of different length have isochronous conduction times, enabling them to activate their synaptic targets simultaneously. Two examples include the projections of retinal ganglion neurons to the lateral geniculate (Stanford, 1987) and projections of inferior olivary neurons to Purkinje cells in the cerebellar cortex (Sugihara et al., 1993). A nearly 2-fold variation in conduction velocity within these myelinated fiber tracts has been reported. The precise mechanism(s) involved have not been established but may reflect axonal diameter, internode length, alterations in node size and, potentially, sodium channel expression—parameters that are all regulated by myelinating glia (see below). Importantly, these findings suggest that myelination may regulate integration of neuronal activity. Modulation of conduction velocity, resulting from changes in axonal diameter potentially regulated by glia, may even occur between proximal and distal regions within a single fiber tract (Baker and Stryker, 1990).

Molecular Composition of Axonal Domains

From the axon hillock to their termination at sensory endings or at presynaptic terminals, ion channels and other membrane components are organized into a series of polarized domains imposed by interactions with myelinating glia. The longest peripheral nerves may have a thousand or more sets of myelin-related domains (e.g., nodes, paranodes, juxtaparanodes, and internodes) along their length. This organization underlies their function in saltatory conduction. In recent years, the molecular composition of each of these domains has begun to emerge (see Figure 2 for summary). A common theme is

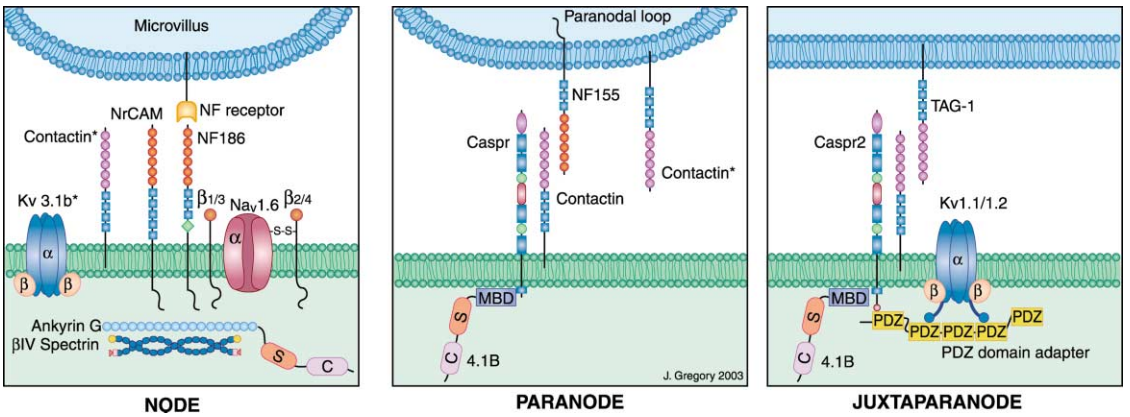

Figure 2. Molecular Composition of Domains of Myelinated Fibers

Major integral and peripheral membrane proteins of axonal domains, and their glial receptors, are illustrated (figure modified from Peles and Salzer, 2000).

Left: Components of mature nodes include NrCAM, neurofascin186, and a trimeric complex of $Na_v1.6$ associated with either β1 or β3 and either β2 or β4. These are tethered to ankyrin G, which binds to a heterodimer of βIV-spectrin. Neurofascin binds in *trans* to a presumptive receptor on Schwann cell microvilli and interacts in *cis* with β subunits of sodium channels. Kv3.1b and contactin are present at some CNS nodes (variable expression indicated by an asterisk). Also present at the node, but not shown, are ECM components including tenascin R, NG2, and brevican; Schwann cell microvilli are enriched in ERMs and EBP50 and actin (not shown).

Middle: Components of the paranodes include a *cis* complex of Caspr and contactin on the axon that binds to 4.1B and interacts with neurofascin 155 on the paranodal loop. In the CNS, contactin is also present on the oligodendrocyte loops and may bind to contactin on the axon.

Right: Components of the juxtaparanodes include a *cis* complex of TAG-1 and Caspr2, which binds to TAG-1 on the glial membrane and interacts with 4.1B in the cytoplasm via a multidomain PDZ protein in the juxtaparanodes, which in turn is linked to $K_v1.1$ and 1.2 on the axon membrane. Juxtaparanodes are also enriched in PSD-95 (not shown).

that multiprotein complexes of cell adhesion molecules and ion channels on the axon bind to cognate ligands on apposed glial processes; these complexes are linked to and stabilized by their association with cytoskeletal elements (Peles and Salzer, 2000). In this section, we review the molecular organization of these domains. Additional details on the components of these domains not covered here may be found in other recent reviews (Girault and Peles, 2002; Kazarinova-Noyes and Shrager, 2002; Scherer et al., 2003).

The Initial Segment and Node of Ranvier

The initial segment and nodes of Ranvier are sites of action potential generation and regeneration, respectively. As befits their physiologic function, both domains are highly enriched in voltage-gated Na^+ channels. Na^+ channels are heterotrimeric complexes of one large, pore-forming α subunit and two transmembrane β subunits, one disulfide-linked and one noncovalently associated (Catterall, 2000). Ten different α subunits have been identified and comprise a multigene family, referred to by a common nomenclature indicated by $Na_v1.x$ (Goldin, 2001). While different Na^+ channels are similar functionally, there are significant differences in their activation kinetics (Yu and Catterall, 2003). $Na_v1.6$ is the predominant channel in adult nodes of Ranvier in both the adult PNS and CNS (Caldwell et al., 2000; Tzoumaka et al., 2000), where it may associate with the β1 subunit (Ratcliffe et al., 2001). Interestingly, the α subunit expressed at the node undergoes a developmental transition (Boiko et al., 2001). $Na_v1.2$ is expressed as nodes form and is later replaced by $Na_v1.6$ in all adult nodes in the PNS and most adult nodes in the CNS (Boiko et al., 2001); a subset of nodes in the adult CNS also continue to express $Na_v1.2$ and $Na_v1.8$ (Arroyo et al., 2002). The physiologic significance of this transition is not yet clear. $Na_v1.6$ is potentiated at high frequencies of activation (W. Zhou and A.L. Goldin, 2002, Soc. Neurosci., abstract) and rapidly recovers from inactivation (Herzog et al., 2003), properties that may enhance its ability to sustain high rates of activity at nodes of Ranvier. In potential agreement, mice deficient in $Na_v1.6$ have slowed nerve conduction velocities (Angaut-Petit et al., 1982; Kearney et al., 2002), although nodal and paranodal abnormalities in these mice may contribute to this slowing (Rieger et al., 1984).

Four β subunit genes have been identified; these differentially modulate the surface expression and regulate the kinetics and amplitude of sodium channel currents (Catterall, 2000; Yu and Catterall, 2003; Yu et al., 2003). β2 and 4 each have unpaired cysteines that are disulfide linked to α subunits (Yu et al., 2003). Both β1 and β2, which associate noncovalently, have been localized to nodes (Chen et al., 2002; Kaplan et al., 2001; Ratcliffe et al., 2001). β subunits each contain a single immunoglobulin (Ig)-like domain and, consistent with this structure, promote homophilic adhesion; they also interact in *trans* with extracellular matrix proteins (Isom, 2002). β1 has also been shown to interact in *cis* with neurofascin and Nr-CAM, which may contribute to the organization of the nodal complex (Ratcliffe et al., 2001). Knockouts of β2 indicate that it is required for full expression and function of sodium channels at the axolemma but not for localization of $Na_v1.6$ at nodes (Chen et al., 2002); the role of other β subunits in channel localization is not yet known.

Na^+ channels are part of a multiprotein complex at the node and initial segments (Peles and Salzer, 2000).

A key component is the multivalent cytoskeletal protein ankyrin G (Bennett and Lambert, 1999), one of three ankyrin genes, each implicated in membrane skeleton organization, targeting, and polarity (Mohler et al., 2002). Ankyrin G has been reported to bind directly to sodium channel α subunits (Bouzidi et al., 2002; Lemaillet et al., 2003) via a conserved motif in the second cytoplasmic loop (Garrido et al., 2003; Lemaillet et al., 2003). Some investigators (Malhotra et al., 2002), but not others (Bouzidi et al., 2002; Lemaillet et al., 2003), have also reported an interaction of ankyrin G with β subunits. Ankyrin G, in turn, binds with high affinity to βIV-spectrin, a novel spectrin isoform that is localized to the node and initial segment (Berghs et al., 2000; Komada and Soriano, 2002). Ankyrin G and βIV-spectrin are required for stable coexpression at nodes and initial segments (Jenkins and Bennett, 2001; Komada and Soriano, 2002). βIV-spectrin provides a presumptive link between the channel complex and the actin cytoskeleton based on classical models (Bennett and Gilligan, 1993), although the spectrin isoform located at nodes may lack the actin binding motif (Komada and Soriano, 2002).

A significant number of additional proteins concentrated at the node also interact with ankyrin, including two neural cell adhesion molecules and at least one potassium channel. NrCAM and the 186 kDa isoform of neurofascin (Davis et al., 1996), both members of the L1 subfamily of IgCAMs (Hortsch, 2000), share a highly conserved cytoplasmic sequence that promotes their interaction with ankyrin G (Bennett and Lambert, 1999) in a phosphorylation-dependent manner (Garver et al., 1997; Tuvia et al., 1997). Neurofascin expressed at the node is not phosphorylated, enabling it to bind to ankyrin G; interestingly, a phosphorylated isoform of neurofascin is enriched in the paranodes (Jenkins et al., 2001), although it appears to be expressed predominately on the glial membranes (Tait et al., 2000). Na^+K^+ ATPase, which binds to ankyrin, has also been localized to the nodal axolemma in some reports (Ellisman et al., 2001) but not others (Gerbi et al., 1999; Kawai et al., 1997).

Recently, Kv3.1b was found to be expressed in a subset of CNS nodes, where it interacts, directly or indirectly, with ankyrin G (Devaux et al., 2003). Its pattern of expression in the spinal cord, i.e., in most nodes of the lateral column but few nodes of the corticospinal tract (Devaux et al., 2003), presumably reflects its differential expression by neurons in the CNS (Rudy and McBain, 2001). Expression of Kv3.1b may contribute to the sensitivity of central nodes to the potassium channel blocker 4-AP (Gordon et al., 1988), although other 4-AP-sensitive potassium channels also appear to be expressed at this site (Devaux et al., 2003). KCNQ2, an M channel that regulates neuronal excitability and repetitive discharges (Cooper and Jan, 2003), was also recently localized to the node (Scherer et al., 2003). Limited expression of K^+ channels has been detected at PNS nodes to date. $K_v1.1$ and 1.2 are transiently expressed during development at PNS nodes, correlating to the temporal pattern of 4-AP sensitivity (Vabnick et al., 1999). Kv3.1b is expressed at a few adult PNS nodes (Devaux et al., 2003). This channel heterogeneity confirms earlier elecrophysiologic studies demonstrating that multiple components of delayed potassium currents are expressed at single nodes of Ranvier (Dubois, 1983).

Initial segments have a similar but not identical molecular composition to nodes of Ranvier. Ankyrin G, βIV-spectrin, Na^+ channels, and ankyrin binding CAMs are all concentrated at initial segments (Bennett and Chen, 2001). Differences in composition of these two domains include the presence of $K_v1.1$ and 1.2 in some initial segments of central neurons but not in their corresponding nodes; conversely, Kv3.1b is in some nodes but not in their corresponding initial segments (Devaux et al., 2003; Dodson et al., 2002). Also, $Na_v1.2$ and $Na_v1.6$ are coexpressed at adult initial segments, whereas nodes principally express $Na_v1.6$ (Boiko et al., 2003). Taken together, these results underscore an unexpected variation in the composition of voltage-gated channels expressed at nodes of different nerve fibers and between nodes and initial segments.

Other nodal components include the glycosyl phosphatidylinositol (GPI)-anchored adhesion molecule contactin, which is expressed at CNS nodes (Rios et al., 2000), where it may associate with β1 and $Na_v1.2$ to enhance channel expression at forming nodes of Ranvier (Kazarinova-Noyes et al., 2001). Gal(β1-3)GalNAc-bearing moieties, an epitope of the GM1 ganglioside, are present at PNS nodes and may be targets of immune-mediated neuropathies (Sheikh et al., 1999). Extracellular components in the CNS include tenascin R, which may stabilize the sodium channel complex by binding to contactin and to the β2 subunit, promoting normal nerve conduction (Weber et al., 1999); versican, a chondroitin sulfate proteoglycan expressed by oligodendrocytes (Courel et al., 1998; Delpech et al., 1982), which may bind to Bral1 synthesized by neurons (Oohashi et al., 2002); and NG2 (Butt et al., 1999; Martin et al., 2001). Proteoglycans are candidates to further stabilize the nodal complex through interactions with components of the axon or glial processes.

Molecular Organization of the Paranodal Junctions

A *cis* complex of the adhesion molecules contactin and Caspr (contactin-associated protein) is a major junctional component of axons (Einheber et al., 1997; Menegoz et al., 1997; Rios et al., 2000). Caspr is expressed by neurons and is tightly associated with contactin, as its acronym implies (Peles et al., 1997); it was independently cloned as paranodin (Menegoz et al., 1997). The extracellular domain of Caspr contains discoidin and fibrinogen-like domains, EGF motifs, and LNS (laminin G, neurexin, slit) domains. This structure places Caspr in a subgroup of the Neurexin superfamily, termed the NCP (Neurexin IV, Caspr, Paranodin) family (Bellen et al., 1998); currently five vertebrate members (Spiegel et al., 2002) and two *Drosophila* proteins, Neurexin IV (Bellen et al., 1998) and axotactin (Yuan and Ganetzky, 1999), are known. Of the vertebrate members, only Caspr interacts with contactin, an association required for efficient transport of Caspr through the endomembrane system to the plasma membrane (Boyle et al., 2001; Faivre-Sarrailh et al., 2000). The essential role of this complex in junction formation was underscored by defects in the paranodes of mice deficient in either pro-

tein, whereas compact myelin forms normally in these mice (Bhat et al., 2001; Boyle et al., 2001). Defects include the consistent absence of transverse bands and abnormalities of adhesion between the paranodal loops and the axon, as evidenced by increased spacing between their membranes and loops that detach and evert over time in the CNS (Bhat et al., 2001; Boyle et al., 2001; Rios et al., 2003). Astrocytic processes in the CNS and microvillar processes in the PNS invade the periaxonal space in the Caspr mutants (Bhat et al., 2001; Rios et al., 2003), indicating that these junctions normally provide a paracellular barrier to soluble diffusion and infiltration of glial cell processes into the periaxonal space. The relative preservation of the morphology of the paranodal region in the PNS but not the CNS in these mutants may reflect additional stability provided by Schwann cell microvilli, basal lamina, and paranodal adherens junctions. These findings suggest that the Caspr-contactin complex is a component of the transverse bands and promotes paranodal adhesion.

In neurons, Caspr targets contactin to the paranodes. Contactin not associated with Caspr is expressed in nodes rather than the paranodes in the CNS (Bhat et al., 2001; Rios et al., 2000); contactin is completely absent from the paranodes of Caspr mutant mice (Bhat et al., 2001; Rios et al., 2000). Caspr has a putative SH3 binding domain and a FERM (4.1, ERM) binding sequence in its cytoplasmic region, suggesting that it functions as a signaling coreceptor for contactin (Menegoz et al., 1997; Peles et al., 1997). Caspr binds to protein 4.1B, a member of the 4.1 family of cytoskeletal proteins (Ohara et al., 2000; Parra et al., 2000), which is enriched in the paranodes and juxtaparanodes (Denisenko-Nehrbass et al., 2003; Gollan et al., 2002; Ohara et al., 2000). Although Caspr and 4.1B have not yet been demonstrated to mediate signaling at the paranodes, evidence that 4.1B functions as a tumor suppressor (Tran et al., 1999; Yu et al., 2002), akin to other 4.1 family members (Sun et al., 2002), supports this notion.

A likely glial ligand for the Caspr-contactin complex is the 155 kDa isoform of neurofascin, which is expressed in the paranodes of myelinating glia (Charles et al., 2002; Tait et al., 2000). NF155 differs in its extracellular sequence from NF186, which is localized at the nodal axolemma (Davis et al., 1996). Consistent with the distinct roles and localization of these two isoforms, neurofascin expression is markedly reduced in the paranodes but not the nodes of Caspr mutant mice (Bhat et al., 2001). A recent report suggests that NF155 binds specifically to the *cis* complex of Caspr and contactin (Charles et al., 2002), although neurofascin was previously shown to bind to contactin directly without Caspr (Volkmer et al., 1998). Contactin is expressed by oligodendrocytes independent of Caspr (Einheber et al., 1997; Koch et al., 1997) and mediates weak homophilic adhesion (Faivre-Sarrailh and Rougon, 1997; Gennarini et al., 1991). These results suggest that contactin detected in CNS paranodes (Rios et al., 2000) may also be expressed by oligodendrocytes where it may bind to neurons homophilically.

In addition to these well-characterized proteins, mice deficient in the myelin glycolipids galactocerebroside and sulfatide exhibit paranodal defects comparable to those of the contactin and Caspr knockout mice (Dupree et al., 1998; Honke et al., 2002). The precise role of these glycolipids in junction assembly is still not understood. They may be required for appropriate trafficking of glial receptors to the paranodes or function as counter-receptors for Caspr via its laminin-G or discoidin domains (Peles and Salzer, 2000; Popko, 2000).

Paranodal Junctions Are Orthologous to Invertebrate Septate Junctions

Septate junctions (SJs) of invertebrates are remarkably similar to paranodal junctions. SJs form a circumferential barrier at the apical portion of the lateral membrane of epithelial cells; the membranes of the two apposed cells are uniformly separated and contain regularly spaced septae, corresponding to an array of parallel strands (Tepass et al., 2001). In *Drosophila*, in addition to epithelial cells, SJs form homotypically between perineural glial cells and heterotypically between perineurial cells and the underlying glia (Carlson et al., 2000).

Many components are common to both junctions. These include neurexin IV, an ortholog of Caspr and Capr2 (see below; Baumgartner et al., 1996; Bellen et al., 1998). NrxIV interacts with coracle, a 4.1 family member, via its juxtamembrane FERM binding sequence (Baumgartner et al., 1996; Ward et al., 1998), and presumptively interacts via its C terminus with discs large (*dlg*), a PDZ (PSD 95, Discs-large, and Z01) domain protein of septate junctions. Orthologs of both neurofascin and contactin, i.e., neuroglian and *Drosophila* contactin, respectively, have also been localized to the SJs (Genova and Fehon, 2003; C. Faivre-Sarrailh and M. Bhat, personal communication). Gliotactin, a novel component that is an ortholog of the neuroligins that bind neurexins at the synapse (Ichtchenko et al., 1995), has an organizational rather than a structural role in SJ formation (Genova and Fehon, 2003; Schulte et al., 2003). Both α and β subunits of the Na^+K^+ ATPase were also recently identified as a crucial component of the SJs (Genova and Fehon, 2003; Paul et al., 2003). Neither Na^+K^+ ATPases nor neuroligins have been identified in the paranodes so far, although these recent reports are likely to spur a reevaluation. SJ components, including coracle, neurexin IV, and neuroglian, form an interdependent junctional complex (Baumgartner et al., 1996; Genova and Fehon, 2003; Ward et al., 1998); loss of one component is associated with mislocalization of all other components (Genova and Fehon, 2003).

Analysis of these SJ mutants has provided insights into the role of these junctions that are relevant to the function of the paranodes. SJs provide a paracellular barrier ("gate" function), which is thought to require the cumulative effect of multiple strands, characteristic of SJs (Lane and Swales, 1982; Tepass et al., 2001). Indeed, NrxIV mutant embryos are paralyzed because this paracellular permeability barrier is required to separate the hemolymph from the nervous system (Baumgartner et al., 1996; Lamb et al., 1998; Tepass et al., 2001). SJ mutants lack transverse septae (Baumgartner et al., 1996) but maintain lateral membrane apposition despite the lack of septae; these results imply that cell adhesion and septae formation are separable functions (Baumgartner et al., 1996; Genova and Fehon, 2003; Lamb et al., 1998). Other junctional components may remain to

promote membrane attachment, a possibility supported by the proper localization of Dlg in SJ (i.e., Cor, Nrg, ATPase) mutants (Genova and Fehon, 2003). However, in gliotactin mutants, these apposed membranes are frequently partially separated, suggesting that the SJs, while not essential, may help maintain membrane apposition (Schulte et al., 2003). SJ components also regulate cell signaling as evidenced by loss of cells in SJ mutations (Ward et al., 1998) and overproliferation of imaginal discs in *dlg* mutations (Woods and Bryant, 1991). Generation of apical and basal lateral domains and overall cell polarity are not affected in SJ mutations (Genova and Fehon, 2003; Schulte et al., 2003), indicating that the septae are not required to maintain a diffusional barrier within the plane of the membrane ("fence" function).

Molecular Organization of the Juxtaparanodes

The juxtaparanodal domain (JPN) lies just under the compact myelin sheath immediately adjacent to the paranodes. It is enriched in two delayed rectifier potassium channels, $K_v1.1$ and 1.2, their β subunits (Rhodes et al., 1997; Wang et al., 1993), and a complex of adhesion molecules that localizes these channels to this site (Figure 2). Specifically, $K_v1.1$ and 1.2 are associated with Caspr2, a neuronal protein that is structurally similar to Caspr but contains, in addition, a binding sequence for PDZ domains at its carboxyl terminus (Poliak et al., 1999). PDZ domain proteins regulate clustering of K^+ channels and glutamate receptors in neurons (Sheng and Sala, 2001). Caspr2 is complexed to $K_v1.1$ and 1.2 through an as yet unidentified PDZ domain protein(s) (Poliak et al., 1999). Although PSD-95 colocalizes and coimmunoprecipitates with Kvβ2 in the juxtaparanodes (Baba et al., 1999), it does not interact directly with Caspr2 (Poliak et al., 1999). Indeed, $K_v1.1$ and 1.2 still accumulate in the juxtaparanodes of mice deficient in PSD-95 (Rasband et al., 2002). Caspr2 also contains a FERM binding sequence and interacts with protein 4.1B at this site (Denisenko-Nehrbass et al., 2003; Poliak et al., 2001).

TAG-1, a GPI-anchored neuronal cell adhesion molecule (Dodd et al., 1988), was recently localized to the juxtaparanodes (Traka et al., 2002), where it is expressed by both neurons and myelinating glia (Poliak et al., 2003; Traka et al., 2003). TAG-1 has a similar domain structure and $\sim$50% identity to contactin (Furley et al., 1990). Caspr2 forms a *cis* complex with TAG-1 (Poliak et al., 2003; Traka et al., 2003) in the juxtaparanodes akin to, but not as tight as, that of Caspr and contactin in the paranodes (Poliak et al., 2003). Recent studies suggest that TAG-1 on the glial cell binds to the *cis* complex of TAG-1 and Caspr2 on the axon via a homophilic interaction (Poliak et al., 2003; Traka et al., 2003). The entire juxtaparanodal complex is dispersed along axons in mice deficient in either TAG-1 or Caspr2, underscoring the key role of this complex in JPN formation (Poliak et al., 2003; Traka et al., 2003). The significance of the localization of juxtaparanodal domain is not yet known, as both knockouts are phenotypically normal and conduction velocities appear to be normal in the Caspr2 mutant mice (Poliak et al., 2003; Traka et al., 2003). Based on individual knockouts of K_v1 channels, they may function to prevent hyperexcitation and backfiring (Zhou et al., 1999). They may also provide a pathway for K^+ ion movement from the axoplasm into the periaxonal space. Interestingly, connexin 29 is precisely localized on the apposing juxtaparanodal glial membrane, where it has been proposed to form hemichannels that remove any excess K^+ in the periaxonal space that accumulates with activity (Altevogt et al., 2002).

Internodal Specializations

By far, the most extended site of interaction between axons and myelinating glia is the internodal region, which comprises nearly 99% of total segment length. The internode is relatively free of specialized structures, of IMPs in freeze-fracture replicas, and of voltage-gated channels (Na^+ channels are present at $<25/\mu m^2$). Nevertheless, it is likely to be an active site of axon-glial interactions, as the separation of the axon and inner glial membrane is a nearly uniform 15 nm, even with significant osmolar changes (Hirano, 1983). MAG is a candidate to mediate these interactions, as it is localized to the inner glial membrane (Trapp, 1990) and binds to sialylated glycoconjugates on the axon (Kelm et al., 1994; Sadoul et al., 1990). A number of neuronal proteins interact functionally with MAG to regulate axonal regeneration (Spencer et al., 2003); it is not yet known whether they contribute to axo-glial interactions in the internode as well.

In the PNS, two specializations are imposed on the axon by noncompacted regions of the Schwann cells: the juxtamesaxon and juxtaincisures. The inner mesaxon of the glial cell is topologically continuous with the lateral edge of the myelin sheath that forms the paranodal spiral. MAG (Trapp, 1990), neurofascin (Tait et al., 2000), and in some fibers, connexin 29 (Altevogt et al., 2002) are enriched on the glial membrane, whereas Caspr and contactin are enriched on the apposed axon surface (the juxtamesaxon). This axonal strand of Caspr and contactin is tightly flanked on each side by Caspr2, TAG-1, and K_v1 channels (Arroyo et al., 1999; Poliak et al., 2003). Thus, the juxtamesaxon appears to be an internodal continuation of the molecular specializations present in the paranodes and juxtaparanodes. As the juxtamesaxonal localization of K_v1 channels persists in the Caspr2 and TAG-1 knockout mice, different mechanisms regulate targeting of this complex to the juxtamesaxon versus the juxtaparanodes (Poliak et al., 2003). The membrane apposing the Schmidt Lanterman incisures, i.e., the juxtaincisures, contain a similar constellation of proteins: a ring of Caspr and contactin on the axon is flanked by TAG-1, Caspr2, and $K_v1.1$ channels. These latter results indicate that the incisures are able to organize the components of the axon despite being separated from the inner glial membrane by a thin collar of cytoplasm (Figure 1).

Local Regulation of Axon Caliber and Transport Rates in the Nodal Region

The polarized organization of the axon is manifest not only in the distribution of membrane proteins, but also in a number of interrelated parameters: the diameter of the axon, the organization of the cytoskeleton, the rate of axonal transport, and the distribution of membranous organelles in the axoplasm (see Table 1). Axon diameter

Table 1. Multiple Parameters Are Polarized in Axonal Domains

	Node	Paranode	Juxtaparanode	Internode
Membrane complex	Na_v, AnkG, Spectrin, NF, NrCAM	Caspr, Contactin, 4.1B	K_v1, Caspr2, TAG-1, 4.1B	?
Axon diameter	reduced	reduced	expanded	expanded
NF phosphorylation	decreased	decreased	increased	increased
Axon transport	reduced	reduced	rapid	rapid
Organelles	increased	increased	slow	slow

Each domain clearly differs based on the components of the plasma membrane and, to a lesser extent, the cytoskeletal scaffold. Other parameters listed (axon diameter, NF phosphorylation, axon transport, and organelle content) appear to be interdependent: the node and paranode show important similarities, as do the juxtaparanode and remainder of the internode.

is locally regulated along the length of myelinated fibers. This is most striking in large PNS fibers, where the diameter of the axon in the paranodal and nodal regions may be reduced to as little as 15%–20% of the internode diameter (Berthold, 1996). This differential regulation of axon diameter is likely to enhance nerve conduction. Large internodal axonal diameters promote rapid nerve conduction (Gasser and Grundfest, 1939; Rushton, 1951; Waxman, 1980); modeling suggests that a reduction in nodal and paranodal diameters would also increase conduction velocity by reducing the surface area and thereby the capacitance of the nodal axolemma (Halter and Clark, 1993).

Axon diameter is increased as a consequence of myelination (Aguayo et al., 1977; Speidel, 1964; Windebank et al., 1985). Axon diameter, in turn, is dependent on the neurofilament cytoskeleton; increased neurofilament content and phosphorylation increases their separation and the diameter of the axon (Ohara et al., 1993; Price et al., 1988; Zhu et al., 1997). Reduced axon diameters in the nodal and paranodal regions may therefore reflect limited radial expansion relative to that of the internode (Aguayo et al., 1977; Raine, 1982) via an effect on the neurofilament cytoskeleton (de Waegh et al., 1992). Indeed, as the result of Schwann cell signals, neurofilaments in unmyelinated regions of the axon, including nodes and paranodes, are reduced in amount and are more ordered and less phosphorylated (Hsieh et al., 1994; Mata et al., 1992; Price et al., 1993; de Waegh et al., 1992) than in the internode; oligodendrocytes similarly regulate these neurofilament parameters in the CNS (Sánchez et al., 1996). Even at Schmidt Lanterman incisures in the internode, the axon diameter is slightly reduced and neurofilaments are more tightly packed (Price et al., 1993).

These results suggest a model in which axo-glial interactions locally regulate a kinase-phosphatase cycle to enhance phosphorylation in the internode and decrease it in the nodal and paranodal regions (de Waegh et al., 1992). One glial molecule that contributes to the radial expansion of the internode is MAG, consistent with its periaxonal distribution and the reduced axon calibers and more tightly packed, hypophosphorylated neurofilaments present in the internode of MAG knockouts (Kumar et al., 2002; Yin et al., 1998). As the diameter of the node and paranodes are still significantly reduced in these mice, other signals must constrain the nodal and paranodal diameters or expand the internodal diameter. Proposed compressive forces exerted by the Schwann cell on the axon in the paranodal region (Zimmermann, 1996) may contribute.

The rate of axonal transport is also markedly decreased in the paranodal and nodal regions. An alteration in rates of transport was first suggested by the disproportionate accumulation of mitochondria, synaptic vesicles, and a variety of other membranous organelles (dense lamellar bodies, multivesicular bodies, and vesiculo-tubular organelles) in the nodal and paranodal regions (Raine et al., 1983; Tsukita and Ishikawa, 1976; Zimmermann, 1996). In some PNS fibers, it is estimated that more than 90% of membranous organelles in the entire axon accumulate in the nodes and paranodes although this region comprises at most 1% of the length of the internode (Berthold et al., 1993). Real-time analysis of organelle transport also supports delayed transport across the node (Cooper and Smith, 1974). In addition, rates of vesicular transport of newly synthesized proteins are also substantially reduced at nodes. Vesicles accumulate proximally and distally, implicating a bottleneck of both anterograde and retrograde transport (Armstrong et al., 1987; Dahlstrom et al., 1992). The role of this accumulation is not known but may provide membrane components, including channels, for assembly and renewal of nodes of Ranvier (Wood and McLaughlin, 1976). They have also been suggested to represent a degradative pathway for organelles from the axon by sequestering them at the node for eventual clearance by the Schwann cell (Gatzinsky et al., 1997).

Slower rates of transport may be a consequence of the significantly reduced diameter of the axon at the node, with accumulation of axonal components akin to that observed in ligated nerves (Berthold et al., 1993). Reductions in transport rates roughly correlates to the extent of axon constriction (Armstrong et al., 1987). Microtubules are also radially constricted in the nodal region (Tsukita et al., 1982), potentially affecting transport rates. Alternatively, in keeping with longitudinal variation in neurofilament (Mata et al., 1992) and neurofascin phosphorylation (Jenkins et al., 2001), local signaling may regulate transport in this region. Vesicular transport involves interactons of cargo with microtubules mediated by members of the kinesin superfamily (Terada and Hirokawa, 2000; see review by Goldstein, 2003, this issue of *Neuron*). Whether kinesin-microtubule interactions are locally regulated or axonal constriction is primarily responsible for reduced transport rates is not known. These findings raise the possibility that axo-glial signaling in the nodal and paranodal regions may coordinately regulate neurofilament phosphorylation, axon transport, and domain assembly.

Epithelial Cell Polarity as a Metaphor for the Organization of Myelinated Axons

Epithelial cells are polarized vis a vis their shape, distribution of membrane proteins and intracellular organ-

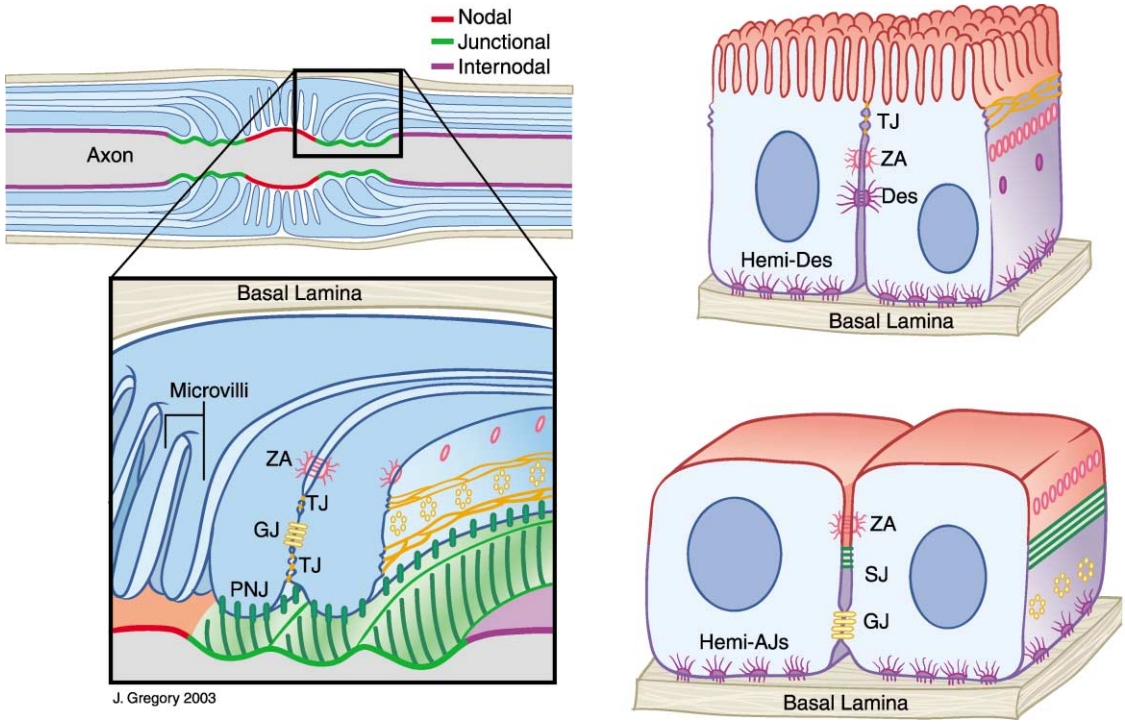

Figure 3. Comparison of the Domain Organization of Myelinated Axons and Polarized Epithelial Cells

Schematic organization of the nodal region of myelinated axons in the PNS is shown on the left and, for comparison, the organization of chordate (upper right) and invertebrate epithelial (lower right) cells. In myelinated fibers, axons are organized into at least three major domains: the node (red), the paranodes (green), and the juxtaparanodal/internodal domain (purple). As shown at higher power in the inset, the axon is continuous with the extracellular space (ECS) at the nodal region and enriched in Schwann cell microvilli. The paranodal region is a site of extensive junction formation including the heterotypic septate junctions, which provide a paracellular barrier between the ECS at the node and the periaxonal space in the internode and separate membrane proteins in the node from those in the juxtaparanode. Paranodal loops form extensive autotypic junctions that are radially and circumferentially arrayed: these include tight junctions (TJ) that provide a presumptive paracellular seal between the periaxonal space and the loops, gap junctions (GJ) that permit direct communication between loops, and adherens junctions (AJ) that promote loop to loop attachment. The apical membrane of epithelia is rendered in red, and the lateral domain, a site of homotypic cell interactions, is rendered in purple. A diffusion barrier between membrane domains is provided by tight junctions in chordates; septate junctions in invertebrates (green), which are orthologous to the paranodal junctions, are interposed between domains.

elles, and the oriented alignment of their cytoskeleton (Knust and Bossinger, 2002). The plasma membrane of polarized epithelial cells is a mosaic of distinct membrane domains, distinguishable on morphologic and molecular criteria. The apical domain faces the extracellular environment and the basal lateral domain contacts the interstitial space (Figure 3). The basal lateral domain is further subdivided into a lateral domain that mediates the adhesion between adjacent cells and provides mechanical support for the epithelium and a basal domain that contacts the substratum. A junctional complex separates the apical and basal lateral domains and regulates the development of polarity (Knust and Bossinger, 2002). In chordates, this junctional complex is comprised of tight junctions (TJs), which separate these domains and the zonula adherens (ZA), and desmosomes, which maintain cell attachment. The tight junctions provide a barrier to paracellular diffusion and to the diffusion of membrane components within the plane of the membrane (Rodriguez-Boulan and Powell, 1992). In invertebrates, as discussed above, the apical and lateral domains are separated by the zonula adherens and septate junctions; the latter provide a paracellular barrier but are dispensible for the separation of apical and basal lateral membrane protein domains and establishment of epithelial polarity. Domains are distinguishable by the presence of three multimeric PDZ protein complexes that assemble in a hierarchical fashion (Bilder et al., 2003; Roh and Margolis, 2003; Tepass et al., 2001).

Neurons are also highly polarized cells, with a single axon and multiple dendrites that differ in their structure and molecular composition (Craig and Banker, 1994). Considerable attention has focused on signals that regulate the formation of the axonal and somatodendritic compartments (see review by Horton and Ehlers, 2003, this issue of *Neuron*). These distinct compartments of neurons develop appropriately in culture, in the absence of spatially organized extrinsic cues, suggesting an intrinsic polarity program (Fukata et al., 2002). They are separated by the initial segment of the axon, which provides a diffusion barrier that impedes lateral mobility of membrane lipids and proteins between compartments rather than a junction with other cells (see below). The axonal compartment has been considered to be an apical equivalent and the somatodendritic compartment a basolateral equivalent based, in part, on the expression of heterologous proteins in the two cell types (Dotti and Simons, 1990). This view has been challenged on mechanistic (Jareb and Banker, 1998) and conceptual (Colman, 1999) grounds.

However, as emphasized here, myelinated axons are not a single uniform compartment, but rather become organized into domains with distinctive membrane and cytoskeletal elements; these are longitudinally and serially organized, rather than columnar. Like epithelial cells, development of polarity in myelinated axons depends on cell interactions, i.e., heterologous interactions with glia. The domains of myelinated axons demonstrate similarities in their organization to that of epithelia (Figure 3). The node, where the axon is accessible to the extracellular space, may be considered a potential apical equivalent. As in apical domains of chordate epithelia, there is also a microvillar collar at the node, in this case contributed by the Schwann cell. The juxtaparanodes and the internode correspond to a putative lateral domain—an extended site of heterotypic cell-cell adhesion. The paranodal junctions, which are functionally and molecularly orthologous to septate junctions of invertebrate epithelial cells, correspond to an intervening junctional complex that provides a barrier between membrane components of the node and internode. The glial paranodal loops themselves resemble a circumferentially arrayed polarized epithelial sheet with radially organized reflexive tight, gap, and adherens junctions (Figure 3). The development of this longitudinal organization of the axon and the circumferential polarity of the glial cell are mutually dependent, as discussed below.

Whether nodes and internodes have molecular complexes characteristic of apical and lateral domains of epithelia, respectively, has not been extensively investigated. In presumptive support, a complex of epithelial sodium channels, ankyrin, and (α) spectrin is targeted to the apical domain of transporting epithelia (Rotin et al., 1994). Also, we have identified several internodal proteins that are expressed in the lateral domain of polarized epithelia (P. Maurel and J.L.S., unpublished data). While much more work will be necessary to clarify the molecular relationship of domains between these cell types, mechanisms of polarization in epithelia provide a useful framework in considering how axonal domains are generated.

Mechanisms of Domain Formation

Establishment of cell polarity requires extracellular interactions that create asymmetries within the membrane and enrich for cell adhesion molecules at sites of cell contact; in turn, these recruit cytoskeletal proteins to which TGN directed vesicles can dock (Nelson, 2003). These mechanisms are supplemented by selective retrieval of mistargeted proteins and stabilization of complexes via extracellular and intracellular interactions (Mostov et al., 2003). Similar principles are likely to regulate assembly of proteins into domains of myelinated axons.

Ion channels and cell adhesion molecules are diffusely distributed on axons cultured in the absence of glia and on ensheathed, nonmyelinated fibers but organize into domains with myelination. These same proteins, although present at lower density, occupy a much greater expanse on unmyelinated and premyelinated compared to myelinated nerve fibers (Salzer, 1995). Thus, total protein levels are typically higher on fibers prior to myelination and are downregulated with myelination; this downregulation may contribute to domain formation by clearing proteins from the internode (see, for example, Einheber et al., 1997). An important question is whether domains form by redistributing proteins present at lower density along the internode, or whether newly synthesized proteins are transported and targeted to their respective domain (Barres, 1998). This question is currently unresolved. Nodes form initially as broad complexes closely associated with the ends of Schwann cells that later appear to fuse and condense (Vabnick and Shrager, 1998). This pattern of clustering, together with the clearance of channels from the internode, has been taken as evidence that nodes form by redistribution of channels at the cell surface via a (diffusion) barrier associated with the end of the Schwann cell (Pedraza et al., 2001; Trapp and Kidd, 2000; Vabnick and Shrager, 1998). Studies in which nodes still formed in transected axons (Rubinstein and Shrager, 1990; Tzoumaka et al., 1995) are consistent with this possibility, although they do not distinguish between proteins at the cell surface or in vesicles within the axoplasm. The alternate possibility, i.e., that clusters assemble by targeting of transport vesicles to nascent nodes, is in potential agreement with accumulation of vesicles in the nodal axoplasm. It is not yet known whether these vesicles are enriched in Na^+ channels known to be transported down the axon (Lombet et al., 1985). This mechanism is likely to account for the transition from $Na_v1.2$ to $Na_v1.6$ well after nodes have formed (Boiko et al., 2001). Indeed, a substantial portion of $Na_v1.6$ staining is intracellular and apparently localized to vesicles associated with microtubules (Caldwell, 2000). Thus, while $Na_v1.2$ may cluster by redistribution from either existing surface or cytoplasmic pools, $Na_v1.6$ is likely to represent newly synthesized protein transported to and preferentially inserted at mature nodes (Salzer, 2002).

Domains assemble sequentially with a characteristic time course. In the PNS, domain assembly progresses from the node to the paranodes to the juxtaparanodes (Melendez-Vasquez et al., 2001; Poliak et al., 2001). This sequence generally correlates with changes in the organization of the Schwann cell, which develops ERM+ processes at either end prior to formation of compact myelin (Melendez-Vasquez et al., 2001). Subsequently, myelin lamellae are elaborated, with their lateral edges forming the paranodal loops; those closest to the node are generated first. Paranodal junctions similarly mature in a sequence beginning with loops closest to the node, progressing inward, (Tao-Cheng and Rosenbluth, 1982). A similar temporo-spatial pattern of Caspr clustering is also observed, with accumulation beginning closest to the nodes (Einheber et al., 1997). Loop attachment may correlate with accumulation of Caspr and contactin in the paranodes, whereas transverse bands, which form later, temporally correlate with neurofascin accumulation in oligodendrocyte paranodes (Marcus et al., 2002). K_v1 channels are transiently expressed in the nodes (Vabnick et al., 1999). Caspr2 and K_v1 are later detected in the paranodes but are displaced into the juxtaparanodes as Caspr and contactin accumulate at this site (Poliak et al., 2001); K_v1 channels persist at reduced levels with Caspr in the paranodes of some spinal cord fibers (Rasband and Trimmer, 2001b). 4.1B appears first in the paranodes and spreads into the juxtaparanodes

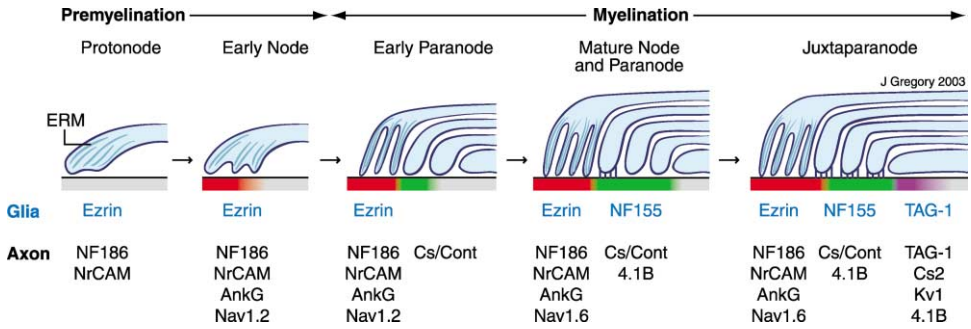

Figure 4. Sequential Assembly and Maturation of Domains in the PNS

A schematic view of the progressive assembly of domains in the PNS. Among the earliest events is the longitudinal polarization of the Schwann cell, indicated by accumulation of ezrin at either end of the Schwann cell and accumulation of ankyrin binding CAMs on the axon in association with these processes (protonode). Shortly thereafter, and typically prior to compact myelin formation, early nodes form, with accumulation of ankyrin G and $Na_v1.2$. During formation of myelin lamellae, Caspr and contactin are first detected in the paranodes closest to the node, but prior to transverse band formation, based on analysis of CNS development (early paranodes). Nodes subsequently mature, with replacement of $Na_v1.2$ by $Na_v1.6$, about the time paranode junctions mature. Finally, juxtaparanodes develop, with accumulation of TAG-1, Caspr2, K_v1 channels, and 4.1B. Juxtaparanodal components, which are present within the paranodes, are displaced into the juxtaparanodes with the accumulation of Caspr (not shown). The sequence of assembly is slightly different in the CNS from that illustrated here (see text for details).

approximately coincident with accumulation of Caspr and Caspr2 at these sites (Poliak et al., 2001). The temporal pattern of domain formation in the PNS is illustrated in Figure 4. The general sequence of events in the CNS is not as well established but appears to be similar; nodes have been reported to cluster prior to the paranodal accumulation of Caspr in some reports (Mathis et al., 2001) although not others (Rasband et al., 1999a). K_v1 channels appear in the juxtaparanodes after a delay of several days (Rasband et al., 1999b).

This pattern of domain assembly could be intrinsically specified by the axon (i.e., inside-out via cytoskeletal interactions) or extrinsically determined by glial signals (i.e., outside-in). Substantial evidence suggests that interactions with myelinating glia drive assembly of axonal domains and determine their location (Peles and Salzer, 2000; Rasband and Trimmer, 2001a). Sodium channels and ankyrin G, which are diffusely expressed on axons cultured in the absence of glia (Ching et al., 1999; Kaplan et al., 2001), cluster only upon direct contact with myelinating Schwann cells (Ching et al., 1999; Kaplan et al., 2001). Soluble factors released by Schwann cells do not cluster sodium channels in tissue culture, although they may in certain pathologic conditions (Deerinck et al., 1997). Similarly, the position of nodes in the PNS appears to be determined by the Schwann cells and is not intrinsically specified. Nodes form in association with either end of myelinating Schwann cells (Vabnick and Shrager, 1998). The length of the Schwann cell is not fixed but rather is variable, progressively increasing during development (Berthold and Nilsson, 1987; Hildebrand et al., 1994). Myelin segments are shorter in certain myelin mutants (Koszowski et al., 1998) and following remyelination (Hildebrand et al., 1994), yet in each case, nodes are appropriately positioned adjacent to the Schwann cell.

A current model of node formation in the PNS is that glial processes interact with and position adhesion molecules, which recruit ankyrin G, thereby creating a site for sodium channel clustering. This model was first suggested by the demonstration that neurofascin and NrCAM accumulate at presumptive nodes prior to ankyrin G and Na^+ channels, which concentrate after a delay (Lambert et al., 1997). These results indicate that nodes assemble locally and suggest CAM recruitment is the initial step. ERM+ Schwann cell processes, which later remodel into the nodal microvilli, directly overlie early nodal intermediates, frequently prior to ankyrin G and Na^+ channels (Melendez-Vasquez et al., 2001) in agreement with earlier ultrastructural studies (Tao-Cheng and Rosenbluth, 1983). ERM+ processes are highly correlated to nodal clustering in vivo (Melendez-Vasquez et al., 2001) and in vitro (Gatto et al., 2003). Defects in Schwann cell microvilli, resulting from ablation of the Schwann cell laminin receptor dystroglycan (Saito et al., 2003) or haplo-insufficiency of the myelin protein P0 (Samsam et al., 2002), are associated with defects of node formation. Blocking the extracellular interactions of NrCAM and neurofascin in myelinating cocultures with a soluble CAM construct that binds to neurofascin inhibits node but not myelin sheath formation (Lustig et al., 2001). In preliminary studies, we have found that neurofascin indeed binds to Schwann cell microvilli in myelinating cocultures (G. Zanazzi, M. Grumet, and J.L.S., unpublished data). As NrCAM knockouts exhibit modest defects of node formation (A.W. Custer et al., 2001, Soc. Neurosci., abstract), neurofascin either partially compensates or plays a primary role in node formation.

A conserved ankyrin binding site on $Na_v1.2$ and $Na_v1.6$ between transmembrane regions II and III (Garrido et al., 2003; Lemaillet et al., 2003) may then target these channels to ankyrin-enriched sites of the axon. This has been directly demonstrated for initial segments and is likely to be true for nodes as well (Garrido et al., 2003). Knockouts of ankyrin G and βIV-spectrin still form nodes but have significantly reduced concentrations of sodium channels at initial segments and nodes, respectively (Jenkins and Bennett, 2001; Komada and Soriano, 2002; Parkinson et al., 2001). Thus, the cytoskeleton appears important for targeting and/or stabilizing components although not demarcating PNS nodes. In the absence

of the nodal cytoskeleton, Na^+ channels may be stabilized at the node by *cis* interactions of their β subunits with matrix components and neurofascin (Ratcliffe et al., 2001; Srinivasan et al., 1998). Taken together, these results suggest that a ligand on the Schwann cell microvilli may concentrate neurofascin on the axon, thereby initiating PNS node development by nucleating ankyrin G sites and/or promoting *cis* interactions with channel components on the axon. Identification of the glial receptor for neurofascin, genetic ablation of neurofascin, and/or inhibition of the microvilli formation will be important to corroborate this model of PNS node formation.

Unexpectedly, CNS nodes and initial segments appear to assemble by different mechanisms than PNS nodes. Nodes form beyond the ends of oligodendrocyte processes (Rasband and Shrager, 2000) but are not contacted by microvilli as they are not elaborated by oligodendrocytes (Melendez-Vasquez et al., 2001). Perinodal glial processes that project to the nodes of large CNS fibers could serve a similar role as microvilli but are not an invariant component of all CNS nodes (Bjartmar et al., 1994). Ankyrin G identifies the earliest nodal intermediates in the CNS, not neurofascin, which is recruited only after a delay (Jenkins and Bennett, 2002). Also, in contrast to Schwann cells, soluble factors released by oligodendrocytes in vitro induce formation of some channel clusters in cultured neurites (Kaplan et al., 1997, 2001). Of note, Na^+ channel clusters induced by soluble oligodendrocyte factors were regularly spaced along cultured nerve fibers, suggesting that they might correspond to nodal intermediates intrinsically specified by the axon (Kaplan et al., 1997). However, in vivo, nodes of optic nerve axons are not regularly spaced along the length of the axon but become progressively more widely spaced farther away from the retina (Baker and Stryker, 1990; Hildebrand et al., 1993). These results may indicate that clusters induced by soluble factors represent "protonodes" that are ultimately repositioned by the glial cell (Salzer, 2002). In addition, if such soluble factors regulate CNS node formation in vivo, they must act over very short ranges given the absence of clustering in the optic nerve head (Boiko et al., 2001) and following partial ablation of oligodendrocytes in vivo (Mathis et al., 2001). Identification of soluble factors released by oligodendrocytes will be important to further clarify their role in CNS node formation.

An alternate mechanism of CNS node formation is that components of the node are actively excluded from the internode, and a diffusion barrier, associated with the ends of the glial cell, concentrates them as the myelin sheath elongates (Vabnick and Shrager, 1998; Pedraza et al., 2001). The notion of a lateral diffusion barrier imposed by the glial cell was originally suggested by the sharp demarcation of nodal intramembranous particles at sites where paranodal loops indent the axon (Rosenbluth, 1976). A second mechanism that might complement a diffusion barrier would occur if paranodal loop interactions induce adjacent ankyrin G clustering that, in turn, functions as a diffusion trap for components of the node. A diffusion trap is believed to promote accumulation of acetylcholine receptors at the neuromuscular junction (Young and Poo, 1983). Accumulation of ankyrin G could recruit channels and CAMs from existing pools of freely diffusing proteins along the axon or, alternatively, serve as a docking site for nodal components that are transported along the axon, as is suggested to occur in the PNS. A critical test of these hypotheses is yet to be carried out.

In contrast to nodes, which require signals from myelinating glia, the initial segment is intrinsically specified and forms in the absence of glia (Alessandri-Haber et al., 2002; Catterall, 1981; Mathis et al., 2001; Winckler et al., 1999; Zhang and Bennett, 1998). This segment may be intrinsically specified owing to its role in neuronal polarity (see below) and its invariant position just beyond the axon hillock; in contrast, the positions of nodes must be continuously remodeled during development, precisely correlated with the longitudinal growth of the myelin segment. Reflecting these differences, ankyrin G and βIV-spectrin accumulate at the initial segment prior to the localization of NrCAM and neurofascin (Jenkins and Bennett, 2001). Loss of ankyrin substantially disrupts initial segment formation but has a more modest effect on node formation (Jenkins and Bennett, 2001; Zhou et al., 1998). Further, as noted, the ankyrin binding sequence of $Na_v1.2$ and $Na_v1.6$ is sufficient to target these and heterologous proteins to the initial segment, underscoring the key role of ankyrin at this site (Garrido et al., 2003).

The initial segment has an emerging role as a diffusion barrier between the axon and somotodendritic compartments (Boiko and Winckler, 2003; Dotti and Poo, 2003). This barrier function has been indirectly correlated to the ankyrin-spectrin cytoskeleton (Nakada et al., 2003; Winckler et al., 1999) and inhibits diffusion of proteins and, remarkably, even phospholipids (Nakada et al., 2003). Potentially, the high density of proteins, including Na^+ channels, that are present at this site act as obstacles to the lateral mobility and diffusion of other membrane components (Nakada et al., 2003). In view of their similarities, these findings raise the possibility that nodes also provide a barrier to diffusion of membrane components into and across the node.

Like PNS nodes, formation of paranodes is dependent on axo-glial interactions (Einheber et al., 1997). Treatment of myelinating cocultures with a soluble RPTP-β/phosphacan construct, which binds specifically to the *cis* complex of Caspr and contactin (Peles et al., 1997), blocks accumulation of this complex at the paranodes without affecting overall myelination (Rios et al., 2000); this complex persisted in many of the internodes of treated cultures. These results suggest that extracellular interactions are required for the proper localization of Caspr and contactin at the paranodes, their downregulation/redistribution from the internode, and normal junction formation. Caspr plays a key role in targeting this complex to the paranodes and junction formation (Bhat et al., 2001). In transgenic mice, Caspr is appropriately targeted to the paranodal region despite deletion of its cytoplasmic domain, but it is aberrantly internalized, suggesting that the cytoplasmic domain is required for stability, not localization (Gollan et al., 2002). Finally, mice deficient in myelin glycolipid synthesis have substantial paranodal abnormalities (Honke et al., 2002; Popko, 2000), providing support for the key role of glial cell interactions in this localization. Taken together, these results suggest that extracellular interactions, potentially mediated by neurofascin on the glial loops

(Charles et al., 2002), promotes assembly of the paranodal complex, which is then stabilized by interactions with cytoskeletal proteins such as 4.1B.

These findings suggest a general model in which axon domain formation is regulated by interactions with myelinating glia (Peles and Salzer, 2000; Rasband and Trimmer, 2001a). In the PNS, progressive differentiation of Schwann cells results in its sequential organization into distinct compartments, which, in turn, drives formation of cognate domains on the axon. Recruitment of adhesion molecules along the axon provide a site to which cytoskeletal proteins assembly and channel proteins can diffuse or dock. Each domain assembles and matures, both morphologically and molecularly, in a characteristic spatiotemporal pattern, suggesting that domains assemble locally rather than as fully formed complexes that simply redistribute during myelination. Extracellular and intracellular interactions within domains stabilize expression of components. The mechanisms of node formation in the CNS may not be driven by juxtacrine signals from apposed glial processes but rather via a glial diffusion barrier in the paranodes and a diffusion trap at the node resulting from the clustered ankyrin G/spectrin cytoskeleton. Future studies should elucidate whether differential sorting of proteins into transport vesicles from the TGN, including the role of the exocyst (Sans et al., 2003), and protein turnover/retrieval of mistargeted proteins, as may occur for sodium channels (Garrido et al., 2001), also contribute to sculpting domains in myelinated axons, supplementing mechanisms described here.

A Central Role for the Paranodes in Domain Organization and Maintenance

Mice with mutations of the paranodal components have been particularly useful to evaluate the role of the paranodes in formation and function of adjacent domains. Although sodium channels still cluster at the nodes of such mutant mice (Bhat et al., 2001; Boyle et al., 2001; Dupree et al., 1999), the function, organization, and maturation of the nodes and the distribution of juxtaparanodal components are aberrant (summarized in Figure 5). As a consequence, nerve conduction velocity and amplitude are markedly reduced (Bhat et al., 2001; Boyle et al., 2001; Dupree et al., 1999); this likely reflects disruption of the paracellular barrier, thereby providing access to the periaxonal space and K_v1 channels. Changes in the node, discussed below, may also contribute to these altered physiological properties.

Glial loops in the paranodal mutants are attached to the axon during the period of node formation and later detach (Marcus et al., 2002; Rios et al., 2003). These initial interactions of the paranodal loops with the axon appear to demarcate the boundaries of the node during development and transiently maintain them in the adult (Rios et al., 2003; Rosenbluth et al., 2003). Thus, central nodes form relatively normally in the Caspr mutant mice but disperse along the axon over time, reaching 7 μm or more in some older mice, presumably reflecting progressive detachment of the paranodal loops (Rios et al., 2003). This dispersion is associated with a proportionate decrease in the concentration of components at the node (Rios et al., 2003). In contrast, nodes are only slightly wider in the PNS where paranodal loops remain oriented toward the axon. These results suggest that paranodal loop interactions, even in the absence of the transverse bands, prevent lateral dispersion of nodal components. This is also consistent with freeze-fracture analyses demonstrating that the boundaries of IMPs are demarcated by paranodal loops, not the transverse septae, during development (Rosenbluth, 1976) and in mutant mice (Rosenbluth et al., 2003).

Interactions of the paranodal loops with the axon, rather than the transverse bands, are also required for the transition from $Na_v1.2$ to $Na_v1.6$ at CNS nodes. Thus, Caspr mutants persistently express $Na_v1.2$ in CNS nodes but appropriately express $Na_v1.6$ in PNS nodes after a delay (Rios et al., 2003). It is not yet known whether the transition in channel subtypes reflects a transcriptional or targeting role of the paranodes. In contrast, the transverse bands are required for the displacement of the Caspr2/TAG-1/Kv1 complex from the paranodes to the juxtaparanodes in both the PNS and CNS (Bhat et al., 2001; Boyle et al., 2001; Dupree et al., 1999; Poliak et al., 2001). Over time, these mislocalized potassium channels clusters are lost in the CNS of paranodal mutants (Ishibashi et al., 2002; Rios et al., 2003). Taken together, these results indicate that paranodal interactions regulate the maturation of the node, including the expression of Na^+ channel subtypes, prevent dispersion of nodal components, and maintain the integrity of the neighboring nodal and juxtaparanodal domains, whereas the transverse bands are required for formation of the juxtaparanodal domain and stable paranodal interactions.

Disorders of Domain Organization and Function in Disease

Disturbances of domain organization and function increasingly appear to be an important source of morbidity in myelin and possibly other neurologic disorders. While an extensive discussion of axon pathology is beyond the scope of this review, several pathogenetic mechanisms will be briefly considered. These include disorders of ion channel localization and expression, disorders of domain components, and axon integrity.

The polarized organization of myelinated axons requires continuous signals from glia (Mathis et al., 2001; Vabnick and Shrager, 1998). However, loss of the myelin sheath does not result in an immediate remodeling of axonal domains. Clusters of Na^+ channels initially persist at sites of former nodes following PNS (Mathis et al., 2001; Rasband et al., 1998; Vabnick and Shrager, 1998) and CNS (Arroyo et al., 2002; Mathis et al., 2001) demyelination. During this time, there are inadequate numbers of intervening channels in the formerly myelinated internode, and nerve conduction is slowed or blocked, contributing to the neurologic disability of MS (McDonald and Sears, 1969). Restoration of conduction requires remyelination or, alternatively, reexpression of sodium channels along the formerly myelinated segment of the axon (Felts et al., 1997), likely involving transcriptional and targeting mechanisms. During the period of remyelination, K_v1 channels are transiently expressed in the nodal region, where they appear to have a significant effect on conduction properties (Rasband

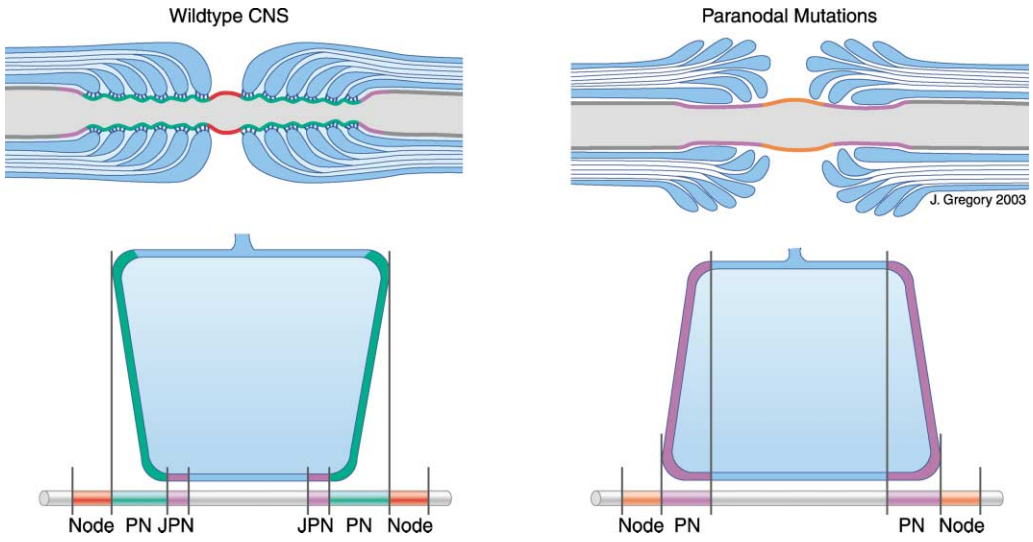

Figure 5. Paranodal Interactions Regulate Domain Organization of Myelinated Axons

Summary of the organization of wild-type and Caspr mutant nerves from the CNS are shown. In the Caspr mutants, juxtaparanodal components of both the axon and glia are mislocalized to the paranodal region, indicating a failure of the barrier (fence) function of the paranodes. Nodes form, but components are present at lower density and over a larger expanse, probably reflecting detachment of paranodal loops and loss of their barrier function. $Na_v1.2$ (orange) persists aberrantly in the nodes of knockouts, indicating a key role of the paranodes in promoting the transition to $Na_v1.6$ (shown in red in the wild-type).

et al., 1998). Subsequently, normal domain organization is reestablished.

In addition to ion channel distribution, myelinating glia also regulate the expression of channel subtypes. Aberrant expression of channel subtypes ("channelopathies") may substantially contribute to disorders in which domains are disrupted. For example, in MS, $Na_v1.8$ expression is upregulated in and results in aberrant physiologic properties of Purkinje cells (Black et al., 2000; Renganathan et al., 2003; Waxman, 2001). A particularly informative example is the transition from $Na_v1.2$ to $Na_v1.6$ at nodes of Ranvier; this likely reflects sequential expression of isoforms during development and the preferential replacement of $Na_v1.2$ by $Na_v1.6$ (Boiko et al., 2001; Kearney et al., 2002). Suppression of $Na_v1.2$ and increased expression of $Na_v1.6$ require signals from myelinating glia (Boiko et al., 2001; Noebels et al., 1991; Westenbroek et al., 1992) that are provided, at least in part, by the paranodes (Rios et al., 2003). In agreement, demyelination from genetic (Rasband et al., 2003) and immune-mediated myelin disorders (Craner et al., 2003) results in the aberrant distribution and expression of sodium channel subtypes. In both instances, nodes are more dispersed and $Na_v1.2$ expression is increased and that of $Na_v1.6$ decreased—similar to abnormalities in Caspr-deficient mice (Figure 5). Paranodal markers are lost earlier than nodal markers following demyelination (Arroyo et al., 2002; Mathis et al., 2001) and may be a sensitive indicator of early myelin abnormalities in MS (Wolswijk and Balesar, 2003). These results suggest that the paranodes may be an early focus of pathogenetic mechanisms that lead to subsequent dispersion of the node and misexpression of channel subtypes.

$Na_v1.6$ has an essential role in nervous system function as evidenced by studies of the *medj* mouse, which does not express this subtype due to a splice donor site mutation (Buchner et al., 2003). These mice have slowed nerve conduction and severe neurologic deficits including motor neuron failure and lethal paralysis (Angaut-Petit et al., 1982; Kearney et al., 2002; Meisler et al., 2001), reflecting a role of $Na_v1.6$ at the nodes and, potentially, in dendrites and muscle where it is also expressed. These mice also have morphologic abnormalities in the node and paranodes that may result from abnormal action potential propagation or an as yet unrecognized structural role of $Na_v1.6$ in node formation (Rieger et al., 1984). Interestingly, Purkinje cells in the *medj* mouse (Burgess et al., 1995) and in ankyrin G-deficient mice (Zhou et al., 1998) undergo degeneration; these results suggest that abnormalities in action potential initiation and/or conduction lead to degeneration of neurons and may contribute to defects observed in myelin-related disorders. Taken together, these findings suggest that aberrant expression of sodium channels at nodes due to defects in paranodal interactions may contribute to the morbidity of demyelinating diseases.

Specific domain components have been identified as targets of autoimmune diseases or are mutated in inherited neurologic disorders. Antibodies to GD1a, which bind preferentially to the nodal region, are associated with acute motor axonal neuropathy (AMAN), a form of Guillain-Barre (Gong et al., 2002). Antibodies to Kv1.1 and 1.2 result in a potassium channelopathy associated with generalized peripheral nerve hyperexcitability (Arimura et al., 2002; Hart et al., 2002). More recently, Caspr2 was found to be mutated in a familial, atypical form of Gilles de la Tourette syndrome (Verkerk et al., 2003). Although the pathogenetic mechanisms are unclear, Caspr2 mutations may result in subtle effects on nerve conduction or repolarization. Whether disorders of nerve conduction, including disruption of isochronicity, under-

lie other neurobehavioral disorders is an important question for future study; provocative associations of myelin-related genes and schizophrenia have been reported (Davis et al., 2003; Tkachev et al., 2003).

Much of the morbidity resulting from myelin disorders reflects loss of axons (Bjartmar et al., 1999, 2003; Martini, 2001; Popko, 2003). This is true not only in acquired disorders of myelin (Trapp et al., 1998), but also in a wide variety of genetic disorders of myelin. Preferential loss of distal axons is a common feature in hereditary neuropathies secondary to Schwann cell defects (Martini, 2001; Sahenk, 1999). The pathogenesis of axon loss in myelin disorders is not yet known (Coleman and Perry, 2002). Immune effects contribute to disease progression, but abnormalities of the axon cytoskeleton, impaired axonal transport, and loss of glial trophic support may also be critical (Bjartmar et al., 2003; Martini, 2001). Abnormalities of neurofilament phosphorylation is a frequent accompaniment of axonal transection in MS (Trapp et al., 1998) and of hereditary neuropathies of myelinating Schwann cells (Martini, 2001). Presumptive evidence of transport defects has emerged from two recent mouse models. Both murine lines have morphologically normal myelin, despite genetic ablation of major myelin-related genes, and they exhibit severe axonopathy and loss. In one, a defect of PLP is associated with a massive increase in vesicles and mitochondria in the node and distal paranode, suggesting a defect in the glial regulation of retrograde transport at this site (Griffiths et al., 1998). More recently, a mouse deficient for the myelin gene CNPase exhibited severe axon loss despite morphologically normal myelin and the lack of an inflammatory response (Lappe-Siefke et al., 2003). These mice also exhibit swellings of the axon that are filled with organelles, suggestive of a defect in transport. These studies indicate that glial trophic mechanisms can be uncoupled from their role in myelination and raise the possibility that other neurodegeneration disorders may prove to have a "myelin" etiology (Popko, 2003). An important focus for future studies will be to identify the trophic signals that regulate axonal transport and sustain its integrity, including whether they are localized to specific domains.

Summary and Future Prospects

This review emphasizes the mutual functional dependence of axons and myelinating glia. Instructive signals from the axon drive the differentiation and reorganization of glia, which, in turn, remodel the axon into a series of polarized domains that differ in their structure, function, and molecular composition. This domain organization exhibits interesting parallels to the columnar organization of epithelia, suggesting that similar principles of domain assembly and establishing polarity may apply. Much remains to be learned about how axonal domains are generated, including the role of TGN sorting and protein turnover and whether multiprotein PDZ complexes identified in epithelial cells also contribute to polarization of axons and/or glia. Presumptive evidence suggests that nodes form differently in the CNS and PNS based, in part, on their sequence of molecular assembly. Current efforts to engineer mutations of key components of these domains should provide significant insights into these questions shortly.

Identification of domain components has been rapid and revealed unexpected heterogeneity in the composition of nodes in different fiber tracts. In particular, several potassium channel subtypes were found to be expressed at a subset of CNS nodes, with more still to be identified. These discoveries provide new targets for therapies aimed at restoration of nerve conduction in demyelinating disorders. This complexity, together with variations in internode length, may provide mechanisms for modulating conduction velocity and presynaptic activity, including synchronizing input, but will require further study to establish their physiologic significance. Local mechanisms that regulate the axon cytoskeleton and axon transport and direct the targeting and expression of ion channels remain poorly understood.

Disorders of axons are now recognized to be a substantial source of morbidity in myelin disorders. Abnormalities in the domain organization of myelinated axons, including disruption of paranodal interactions, provide new ways to conceptualize these disorders and are certain to spur new insights into their mechanisms. Defective interactions of axons with myelinating glia may also prove to underlie neurodegenerative and neurobehavioral disorders not traditionally appreciated to be "myelin" disorders.

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